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CHRONIC INHALATION TOXICITY OF UNSYMMETRICAL DIMETHYLHYDRAZINE: ONCOGENIC EFFECTS

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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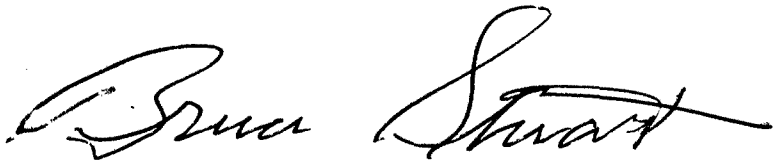

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Four animal species were exposed for 6-months to selected vapor concentrations of propellant grade unsymmetrical dimethylhydrazine to determine its oncogenic effects. The UDMH concentrations at or near the current threshold limit value were 0.05, 0.5, and 5 ppm. Animals were maintained for various time periods postexposure as long as: hamsters, 17 months; mice and rats, 19 months; and dogs, 54 months. The lung was a target organ in rats while the liver was the main target of 5 ppm UDMH induced neoplastic changes in rats and mice.

Rats had an increased incidence of bronchiolar adenomas compared to controls. Mice exposed to 5 ppm UDMH developed a 4.7% incidence of liver hemangiosarcomas while controls had a 0.7% incidence. Kupffer cell sarcomas were also increased in UDMH exposed mice.

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BLOCK 19. Abstract

An indication of hepatotoxicity in dogs was revealed by transitory elevation in SGPT and BSP values for dogs exposed to 5 ppm. Since the UDMH used in this study contained approximately 0.12% dimethylnitrosamine (DMNA), the possibility that this hepatotoxin was responsible for the liver effects in dogs was examined by a series of short-term repeated exposures of dogs to 5 ppm UDMH with 0.12% DMNA and UDMH without DMNA. Dogs that received UDMH containing DMNA showed significantly increased SGPT serum levels while those exposed to UDMH alone showed no increases indicating that DMNA was the hepatotoxic agent. As a companion study, mice were given repeated oral doses of DMNA equivalent to various calculated inhalation concentrations. Oral doses of DMNA equivalent to and twice the concentration of DMNA in the highest concentration of UDMH used in the 6-month study did not produce significant pathological changes in mouse livers during and following repeated doses over a 60-day period.

In an attempt to resolve the uncertainty as to the chemical agent responsible (UDMH or DMNA) for tumor production in the 6-month study, mice only were exposed to 5 ppm purified UDMH for 12-months followed by a 12-month latency period to allow for tumor induction. Alveolar/bronchiolar adenomas were excessive in exposed mice, 10.8% compared to 2.1% in controls. There was a 10.6% incidence of hepatocellular adenomas in exposed mice and 2.1% in controls. Malignant lymphomas, 44.2% in exposed mice and 33.5% in controls. Nasal papillomas and adenomatous polyps, 2.8% and 9.5%, respectively in exposed animals and none in controls. Angiomatous tumors (hemangiomas and hemangiosarcomas) 15.3% versus 5.2% in controls. Approximately 50% of these tumors in exposed mice were found in the liver.

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document constitutes a final technical report on UDMH. The research covered in this report began in September 1974 and was completed in June 1980 and was performed in part under Air Force Contract No. F33615-76-C-5005 and F33615-80-C-0512. K. C. Back, Ph.D. and M. K. Pinkerton served as contract technical monitors for the Air Force Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D. served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor and Chairman, Department of Community and Environmental Medicine. Acknowledgement is made to D. L. Pollard, J. D. Diaz, J. D. Collins, A. K. Roychowdhury, Ph.D., C. D. Flemming, and J. C. Welch for their significant contributions and assistance in the preparation of this report. Acknowledgement is also made to Maj K. M. Ayers and Capt G. B. Baskin, formerly of the Air Force Aerospace Medical Research Laboratory, and to Lt Col W. R. MacKenzie, Ret., Lt Col R. E. Schmidt, Ret., and Maj R. L. Eason, Ret., formerly at the USAF School of Aerospace Medicine, Brooks Air Force Base, Texas, for their assistance in the evaluation of pathologic changes in animals.

The Committee on Toxicology, NAS-NAE/NRC (1974) recommended that further research on the potential carcinogenicity of hydrazines should be conducted. Because of this, along with the evidence in the literature of UDMH-induced tumorigenicity in animals and the demonstrated oncogenic effects of a closely-related compound, hydrazine, in mice following a 6-month inhalation study (MacEwen and Vernot, 1974), experiments were carried out to characterize carcinogenicity of UDMH including dose response. Results obtained during and at the completion of the first studies led to subsequent experiments to test hypotheses generated by the earlier exposures.

Section II of this report presents the results of the principal study of the effects of inhaled UDMH while Section III deals with the hepatotoxic response of beagle dogs to UDMH with and without DMNA and the peroral toxicity of dimethylnitrosamine in mice. Section IV provides the rationale for and the results of a study of the oncogenic potential of inhaled purified UDMH in mice.

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SECTION I

INTRODUCTION

UDMH (unsymmetrical dimethylhydrazine) is 1,1 dimethylhydrazine also known as asymmetrical dimethylhydrazine and dimazine. This compound is a strongly reactive alkaline liquid which is commercially produced by the reduction of dimethylnitrosamine or by the reaction of dimethylamine with chloramine. Although it is used in photographic chemicals, chemical synthesis and as a plant growth control agent, the major use and production is for rocket propulsion either alone or in combination with other hydrazines. Aerozine-50 is a missile fuel consisting of an equal mixture by weight of UDMH and hydrazine. This combination along with nitrogen tetroxide as the oxidizer was used in some of the Apollo flights. UDMH was also used to propel the Titan group of boosters used in the Gemini program.

The structure and physical properties of UDMH are shown below:

$(CH_3)_2NNH_2$ = 1,1-dimethylhydrazine

Molecular Weight:	60.1
Liquid Density:	0.784 g/cc at 25°C
Boiling Point:	63°C
Critical Temperature:	250°C
Flash Point (Closed Cup):	1.1°C
Vapor Pressure:	15.5 mm Hg at -17.8°C
	51.8 mm Hg at 4.4°C
	160.5 mm Hg at 26.7°C
	435.0 mm Hg at 48.9°C

BACKGROUND

Five reviews of the toxicology of the hydrazines, as a group, have been published in the past 16 years. All hydrazines demonstrate considerable toxicity. Clark et al. (1968) provided a thorough dated accounting of the toxicology and pharmacology of the hydrazines. This was followed by a review by Back and Thomas (1970). The Committee on Toxicology, National Research Council,

National Academy of Science published a comprehensive review of the health hazards from hydrazine, monomethylhydrazine, and UDMH four years later (1974). Continuing concern was reflected in a subsequent article by Back et al. (1978) dealing with pertinent information collected since 1970. The NIOSH Criteria document (1978) appears to be the most current and extensive review of the toxicology and particularly the carcinogenicity of the hydrazines.

HUMAN EXPOSURE

Reports of human exposure to UDMH are scant and not informative as to exposure levels or long-term effects, including carcinogenicity. Shook and Cowart (1957) reported the intermittent exposure of five laboratory workers during a six month period. During the first 3 months the men worked 10 hours/day, 6 days/week, then infrequently (6-8 times) for no more than 4 hours/week during the next 3 months. Additionally, 6 men engaged in transferring UDMH from 55 gallon drums to storage tanks had 3 to 4 days of outside exposure to UDMH during a 6 month period. One accidental spill did occur during this time. Positive cephalin flocculation tests were noted for six of the eleven workers during or after the episodes. However, there were no other signs of toxicity. Petersen et al. (1970) found elevated SGPT levels in Danish Air Force personnel who worked with liquid rocket propellants which included UDMH in unknown concentrations. Measurements were made 3-4 times over a 3 year period. SGPT activity was elevated at least once in 47 of 1,193 persons examined. Liver biopsies from 26 volunteers revealed some form of fatty degeneration in 10 cases. High SGPT values were noted in all cases where deposition was found. Although no other conditions known to cause liver damage were identified in the subjects, it was not possible to confirm that the hepatic effects were exclusively due to UDMH exposure.

INHALATION AND DERMAL STUDIES

There have been many investigations of the toxicity of UDMH given by several routes of administration to a variety of experimental animals. Early work consisted mostly of acute and subacute studies designed to produce data on short-term effects in order to set standards for worker protection against inhalation exposure and cutaneous absorption. Acute toxicity information, from studies using other forms of dosage can be found in the review references cited previously. Jacobson et al. (1955) reported the death of 2 of 3 dogs following a 4-hour inhalation exposure to 111 ppm. All three dogs exposed 4-hours at 24 ppm survived, but one vomited and convulsed. Their blood counts, bromsulphalein retention, and

prothrombin times were normal. Four hour LC_{50}'s for mice, rats and hamsters were calculated to be 172, 352, and 392 ppm, respectively. Convulsions and respiratory tract irritation were seen in many of the rodents.

Weeks et al. (1963) exposed rats and mongrel dogs to various UDMH concentrations for brief time periods and obtained LC_{50} values for dogs of 22300, 3580 and 981 ppm and for rats of 24500, 8230 and 1410 ppm, for 5, 15, and 60 minutes, respectively. Serial sacrifice and microscopic examination of rats that survived for as long as 21 days postexposure revealed no changes attributed to UDMH. Groups of 4 dogs each were exposed twice a week for 6 weeks to 50, 200, and 600 ppm for 60, 15, and 5 minutes, respectively. There were no hemolytic effects or abnormal changes in other blood measurements. Conditioned avoidance tests were also conducted, but there were no significant changes in behavior. As part of the study, Weeks also determined the retention of inhaled UDMH in pentobarbital anesthesized dogs. Each of 5 dogs were exposed for approximately 60 minutes by means of a face mask or endotracheal tube. Inhaled doses of 2.9 to 19.6 mg/L showed retention of 71 to 93%.

Rinehart et al. (1960) exposed one group of rats and mice to 140 ppm for 6 weeks and another group to 75 ppm for 7 weeks. Two groups of 3 dogs were exposed to 25 ppm for 13 weeks or 5 ppm for 26 weeks. All exposures were for 6 hours/day, 5 days/week. At 140 ppm, 29 of 30 mice and 1 of 20 rats died. At 75 ppm, 22 of 30 mice and all of 30 rats survived. Tremors were a consistent sign in the exposed mice, and those that died had tonoclonic convulsions. One dog died during the 25 ppm exposure, while none succumbed to 5 ppm. Severe CNS effects were noted during exposure to 25 ppm while only mild signs of toxicity were observed in the dogs that received 5 ppm. Dose-related weight loss and hemolytic anemia were evident in both groups of dogs. When first measured, after 4 weeks of exposure to 25 ppm, RBC counts were decreased by 58%, hematocrit values by 28%, and hemoglobin concentrations by 34%. Subsequent hematocrit and hemoglobin measurements showed recovery to near normal values, but RBC counts remained depressed. Bromsulphalein retention time, bilirubin, blood glucose and nonprotein nitrogen levels were all normal. After 24 weeks, dogs exposed to 5 ppm showed 17%, 18%, and 26% decreases in RBC, hematocrit, and hemoglobin values, respectively. Pathologic examination of the rodents showed no tissue alteration that was due to UDMH. Dogs that survived the 25 ppm exposure showed hemosiderosis in the cells of the reticuloendothelial system that was most prominent in the liver; but was also seen in the spleen, lymph nodes, and bone marrow. Bone marrow demonstrated significantly increased erythrocytic activity. Lung tissue of the dog that died during exposure showed alveolar hemorrhaging, emphysema and atelectasis, but no hemosiderosis. Dogs exposed at 5

ppm for 26 weeks showed hemosiderosis only in the spleen; no other abnormalities were in evidence. The authors concluded that the dog was the most sensitive species tested. The occurrence of hemolytic anemia at low doses was sufficient to keep industrial UDMH concentrations well below 5 ppm. They recommended 0.5 ppm as a guideline for safe industrial practice.

In addition to the inhalation hazards, dermal and ocular contacts represent primary routes of worker exposure to UDMH. For this reason percutaneous absorption and effects from the instillation of UDMH in the eyes of animals were investigated. Rothberg and Cope (1955) determined LD_{50}'s of 1.05 g/kg for rabbits and 1.31 g/kg for guinea pigs by skin absorption. There was corrosive damage to the skin. Only mild irritation was seen in the eyes of 2 rabbits dosed with 3 µl. Hodge (1954) instilled about 0.05 ml into one eye of a rabbit and saw only slight vascularization of the eyelids. He also applied UDMH to the clipped abdomens of rabbits. It was lethal at 156 mg/kg and nonlethal at 23 mg/kg. Smith and Clark (1971) spread doses of 30-1800 mg/kg on the shaved chests of 13 anesthesized mongrel dogs. There were no signs of skin damage, but 6 of the dogs died about 6 hours after the application. Convulsions were seen in 3 dogs. The LD_{50} was estimated to be 1200-1680 mg/kg.

TUMORIGENICITY

Investigations of the tumorigenicity of UDMH have been limited to oral dosing and intraperitoneal injections. Toth (1973) described tumor formation in groups of 50 male and 50 female Swiss mice provided with 0.01% solution of UDMH in drinking water for life, an average daily intake of 0.7 mg. The UDMH ingestion significantly shortened the survival time of the treated mice compared with the untreated controls. Blood vessel tumors were seen in 74% of the female and 84% of the male mice at an average age of 59 and 42 weeks, respectively. The tumors were characterized as angiosarcomas found predominantly in the liver. Lung tumor incidence was 64% in females and 78% in males at average ages of 62 and 53 weeks. The vast majority of tumors were adenomas although 6 females and 4 males had adenocarcinomas. One female and 9 male mice developed kidney tumors at 60 and 59 weeks of age. Benign hepatomas were seen in 6 males at 58 weeks. Malignant lymphomas were observed in 7 females and 1 male at average ages of 55 and 60 weeks. The overall tumor incidence was 79, 71, 10 and 6% in the blood vessels, lungs, kidneys and liver, respectively. The majority of the blood vessel tumors were in the liver. Corresponding incidences in the controls were 2, 11, 0, and 0%. Roe et al. (1967) intubated a group of 28 female Swiss mice with UDMH doses of 0.5 mg/day, 5 days/week, for 40-60 weeks. During this time, serial sacrifices and necropsies

of some of the treated and untreated mice were conducted. Lung adenomas or adenocarcinomas were seen, and because of multiple tumor formation in the treated group, the authors concluded that this fact supported the view that UDMH is tumorigenic. However, the overall incidence of tumors in the treated group was not statistically greater than incidence in the control group. Kelly et al. (1969) gave a group of 30 CDF-1 male mice, aged 7-8 weeks, 8 weekly i.p. injections of 120 mg/kg UDMH. In addition, 30 female mice were given 8 oral doses of 277 mg/kg. Because the incidence of lung tumors seen at 28-32 weeks was far less than that for controls, the authors concluded that UDMH was not carcinogenic in mice.

Since dimethylnitrosamine (DMNA) is used in the production of UDMH and may remain in the final product, a review of the pertinent toxicology of DMNA is appropriate. Nitrosamines as a class are known to be hepatotoxic and carcinogenic in animals (Magee and Barnes, 1967). Although there is no known instance at this time of cancer production in humans as a result of DMNA exposure, this compound has been found to be highly hepatotoxic and carcinogenic in all species of animals tested. DMNA hepatotoxicity was first reported in humans and animals by Freund in 1937. A young chemist who synthesized DMNA survived after a long illness and another worker died after a single exposure to an accidental spill. Freund conducted laboratory exposures to DMNA using dogs and mice after observing those human episodes and found liver damage resembling that seen in the two patients. Other cases of nonfatal liver injury in humans have been described by Barnes and Magee (1954) and Jacobson et al. (1955), and prompted their animal toxicity studies. Severe liver necrosis and death was caused in rats, rabbits, mice, guinea pigs, and dogs as a result of acute doses of 20 to 40 mg/kg of DMNA (Barnes and Magee, 1954). Jacobson and coworkers (1955) exposed mice, rats, and dogs to various vapor concentrations of DMNA for 4 hours and produced LC_{50}'s of 57 and 78 ppm for mice and rats, respectively. A concentration of 16 ppm killed 2 of 3 dogs. Liver damage was a common finding in all species. Druckrey et al. (1964) provided evidence that DMNA is carcinogenic in animals by the inhalation route. Exposure of rats to 100 ppm for one half hour per week produced carcinomas of the ethmoid cells of the paranasal sinuses. Several oral studies on different strains of mice have shown tumors in the form of hemangiomas, hemangioendothelial sarcomas, hemangioendotheliomas, adenomas, and hepatocellular carcinomas of the liver, as well as adenomas and adenocarcinomas of the lung (IRAC Monographs, 1972).

The lowest dose given to mice in a chronic study was a daily ingestion in the drinking water of 0.4 mg/kg body weight (Clapp and Toya, 1970). The total dose of 89 mg/animal increased the incidence of lung and liver tumors above control values. The lowest dose

given to rats was 2 ppm in their diet (Terracini et al., 1967). Concentrations used in this study ranged from 2 to 50 ppm. At 2 and 5.0 ppm, the frequency of tumors among survivors at 60 weeks were 1 of 26 and 8 of 74. Liver tumors were seen in more than 66% of the rats that received 20 or 50 ppm. A concentration of 2 ppm in the diet for a year corresponds to a total intake of 22 mg/animal. The lowest dose given to hamsters resulted from weekly subcutaneous injections of 0.5 mg for 4 to 5 months (total dose 8-11 mg/animal) (Herrold, 1967). This treatment caused hemangioendothelial sarcomas and cholangiocarcinomas of the liver and neuroepithelial tumors of the posterior nasal cavity. No reports of studies to determine carcinogenic potential of DMNA in dogs were found in the literature.

The absence of epidemiologic information and an established TLV indicates that DMNA has not been an industrial hygiene problem although it is suspect of carcinogenic potential in man (TLV A2 notation) by the ACGIH; and considered as a carcinogen by the FDA.

SECTION II

A SIX-MONTH CHRONIC INHALATION EXPOSURE OF ANIMALS TO UDMH TO DETERMINE ITS ONCOGENIC CAPACITY

A combined chronic inhalation toxicity and oncogenicity study was undertaken with four animal species. In this study beagle dogs and three rodent species, rat, mouse, and hamster, were exposed to UDMH vapors on an industrial type 6-hour daily regimen for six months. The animals were held for an additional 18 months in the case of rodents and a total of 5 years for dogs.

MATERIALS AND METHODS

Animals

The number of animals used in this experiment was selected to permit a statistically valid number of rodents to reach the required age for tumor induction with natural and toxicologic attrition. Dogs were selected because they are the most sensitive laboratory animal to non-carcinogenic chronic effects of the hydrazines (hemolytic and CNS) and are also the most suitable species for monitoring hematologic and biochemical status during the exposure period. Periodic testing of hepatic function in the dogs would also provide the data base for determining the capability of UDMH to produce liver damage.

Animals used in exposed and control groups were 400 female C57BL/6 mice obtained from Jackson Laboratories; 200 male (CDF®[F-344]/CrlBR) rats from Charles River Breeding Laboratories; 200 male Golden Syrian hamsters from Engle Laboratory Animals, Incorporated; and 4 male and 4 female beagle dogs from Ridgelan Farms.

We were unable to initiate the exposures to all dose levels simultaneously because of the number of chambers needed. Therefore, a separate set of controls was provided for the low dose group with this part of the experiment started three months after the initiation of the intermediate and high dose exposures. Hamsters were added even later to the low dose exposure because animals initially processed failed to pass quality control examination. These hamsters were 26 months younger at the start of exposure than those exposed to higher levels of UDMH.

Exposure Conditions

The exposure concentrations of 0.05, 0.5 and 5 ppm were selected to include the ACGIH Threshold Limit Value of 0.5 ppm and to provide a wide range of concentrations spanning those with minimal effect to maximum tolerated concentrations.

The exposures were conducted on a 6 hour/day, 5 day/week schedule for 6 months without exposures on holidays. Two chambers were used for each UDMH concentration. Each pair of chambers contained as few species as possible to minimize the risk of cross infection. Dogs and rats were housed in one chamber and mice and hamsters in the companion chamber. All control animals were maintained in animal holding facilities. The chambers were operated with nominal airflows of 35 cfm at a slightly negative pressure of 725 mmHg to prevent leakage of UDMH into the laboratory. The airflow, pressure, relative humidity, and temperature in each chamber were controlled automatically. Relative humidity was maintained at 50% ± 10% and the temperature at 22°C ± 2. The 6 month animal exposures were followed by a near lifetime observation period for potential tumor induction and detection of residual toxic effects. Dogs were held 5 years.

UDMH Generation and Analysis

The UDMH used in this experiment was propellant grade obtained from the United States Air Force Rocky Mountain Arsenal. UDMH was introduced into the air supply system of the exposure chambers by means of syringe feeders. It was metered into heated lines where it was vaporized and blended into the air stream. Exposure chamber

concentrations of UDMH were controlled either by changing the delivery rate of the syringe feeder or by small adjustments of the airflow rates or both. The UDMH vapor concentrations were monitored using an AutoAnalyzer and an iodometric method described by Geiger (1967). An analyzer system was operated for each pair of chambers at the same concentration. Automatic switching valves allowed the analysis of air samples from alternate chambers at 10 minute intervals. The air from each chamber was sampled continuously with separate absorber columns with switching of the absorbing solution flow into the colorimeter at 10 minute intervals.

Clinical Tests and Observations

All animals were observed hourly during the 6-month exposure phase of the study and daily during the postexposure phase for signs of UDMH intoxication and mortality. Rats, hamsters, and dogs were weighed individually at biweekly intervals during exposure and monthly during the postexposure period. Mice were weighed in groups and group mean weights were recorded on a monthly basis throughout the experiment.

Blood samples were drawn from dogs at biweekly intervals during exposure and at various times postexposure. Clinical determinations were made for the following battery of tests:

RBC	Glucose
WBC	Total Protein
HCT	Albumin
HGB	Globulin
Sodium	A/G Ratio
Potassium	SGPT
Calcium	Alkaline Phosphatase

Blood measurements not included in the regular biweekly schedule during the exposure phase of the study but made at the conclusion of the 5.0 and 0.5 ppm UDMH experiments were:

Blood Urea Nitrogen	SGOT
Chloride	Prothrombin Time
Cholesterol	Cephalin Flocculation
Creatinine	Bromsulphalein

Of these, tests giving abnormal values were repeated postexposure at selected intervals until recovery. Additionally, to examine

for possible hemolytic effects in rodents, blood samples for hematocrit and red blood cell counts were taken from 5 rats and 5 hamsters from each group at the conclusion of the 5.0 and 0.5 ppm exposures. Blood was withdrawn from the suborbital region of the eye using a nondestructive technique.

All animals that died or were killed during the study were necropsied following the protocol for microscopic tissue examination used by the National Cancer Institute biocollaborative carcinogenesis research program (1976). The necropsy consisted of an external examination including all body orifices, and the examination and fixation of portions of organs or tissues for histopathologic examination.

Body weight, hematology, and clinical chemistry data were entered into computer files for automated data processing. Significance of differences in group means was tested using Student's "t" test. Difference in incidence rates of histologic lesions including tumors were evaluated using the Fisher Exact Test or trend analysis.

The evaluation of histologic specimens from hamsters was performed by the Veterinary Sciences Division at the USAF School of Aerospace Medicine at Brooks Air Force Base, Texas. Tumor and nontumor nomenclature was developed and used by this group for automated data processing of incidence rates. The nomenclature used for rats, mice, and dogs was a standardized system called MINISNOP (miniaturized systematized nomenclature of pathology). Use of this system allows comparisons of lesion incidence rates among species and among pathologists.

RESULTS

Concentration Measurements

The nominal desired concentrations of 0.05, 0.5, and 5.0 ppm UDMH were maintained in paired chambers throughout the 6-month exposure phase of the experiment. Monthly mean concentrations obtained by continuous analysis for each month of inhalation exposure chamber operation are shown in Table 1 along with the overall means for each chamber. The desired mean UDMH concentration of 0.05 ppm was exceeded in chambers 7 and 8 during the first month of operation beyond a desired limit of 10%. However, excursions thereafter were within 10%. All other chamber concentrations were maintained within this limit for the duration of exposure. Because some species were added to the study after others, the calendar months shown in Table 1 exceed six; however, individual animals received only 26 weeks of exposures.

TABLE 1. MEASURED MONTHLY MEAN CONCENTRATIONS OF PROPELLANT GRADE UDMH IN THE ANIMAL EXPOSURE CHAMBERS

Calendar Month	Chamber 1[a] 5.0 ppm	Chamber 4 5.0 ppm	Chamber 5[a] 0.5 ppm	Chamber 6 0.5 ppm	Chamber 7 0.05 ppm	Chamber 8[b] 0.05 ppm
1	5.01	5.03	0.50	0.53	0.062	0.067
2	5.03	5.02	0.51	0.49	0.052	0.053
3	4.99	5.03	0.49	0.54	0.052	0.049
4	5.30	5.01	0.50	0.46	0.051	0.052
5	4.97	4.91	0.49	0.54	0.055	0.055
6	5.00	4.94	0.52	0.51	0.047	0.047
7	5.00	5.03	0.53	0.52	0.045	0.045
8	4.96	----	0.49	----	-----	0.047
9	----	----	----	----	-----	0.050
Overall Mean	4.91	5.00	0.50	0.51	0.052	0.052

[a] Chambers 1 and 5 operated longer to complete exposures of mice started later than those for other species.

[b] Chamber 8 operated longer to complete exposures of hamsters started later than those for other species.

Animal Weights

The experimental animals were weighed biweekly during exposure and monthly postexposure. The mean weights for rats are shown in Figure 1 and for mice in Figure 2. Mean weights for the hamsters are presented in Figure 3. All groups of rats and hamsters gained weight during exposures at slightly lower rates than their unexposed controls. The differences were statistically significant in most cases, but not dose related. One group of unexposed control rats housed in a separate animal holding room were afflicted with a severe respiratory infection that caused deaths of approximately 25% of the group and significant weight loss in the survivors. These rats had weights below both the UDMH exposed rats and a second set of controls throughout the remainder of the study. After statistical examination of the data showed gross variations between the two unexposed control rat groups the aberrant group was removed from further consideration in the study and all subsequent analysis and comparisons of growth, clinical measurements, and pathology were made with the good control group of rats. Growth of the mice and the dogs was unaffected by UDMH exposure.

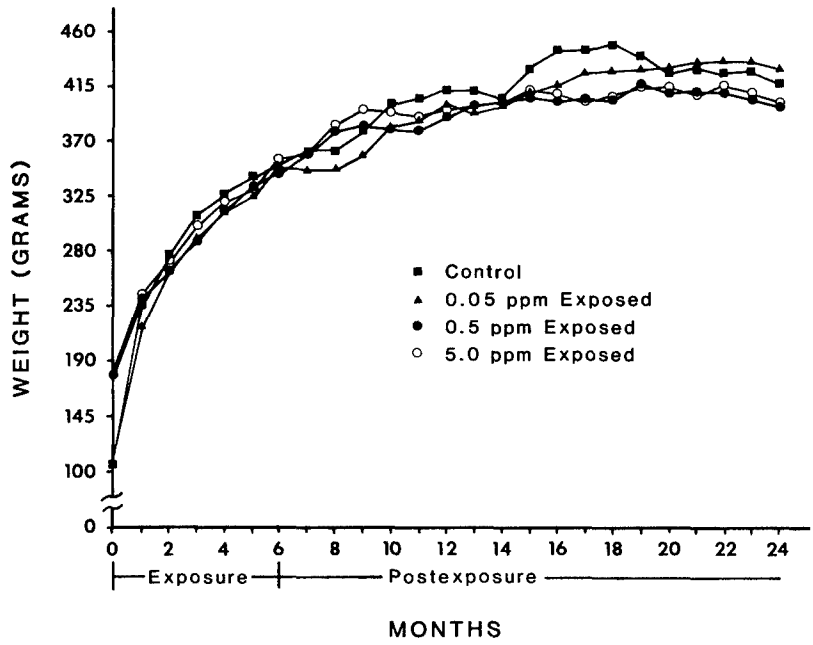

Figure 1. Effect of chronic exposure to inhaled Propellant Grade UDMH on the growth of male Fischer 344 rats. (Group mean weights)

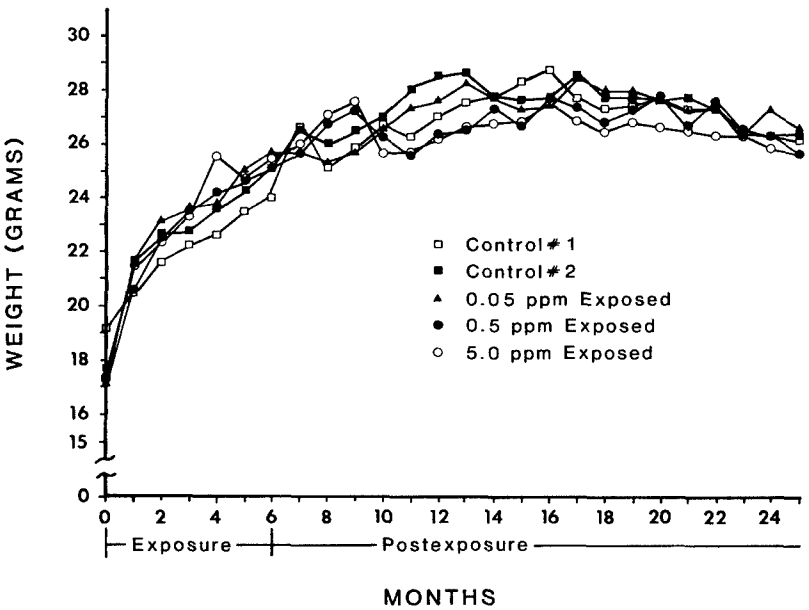

Figure 2. Effect of chronic exposure to inhaled Propellant Grade UDMH on the growth of female C57BL/6 mice. (Group mean weights)

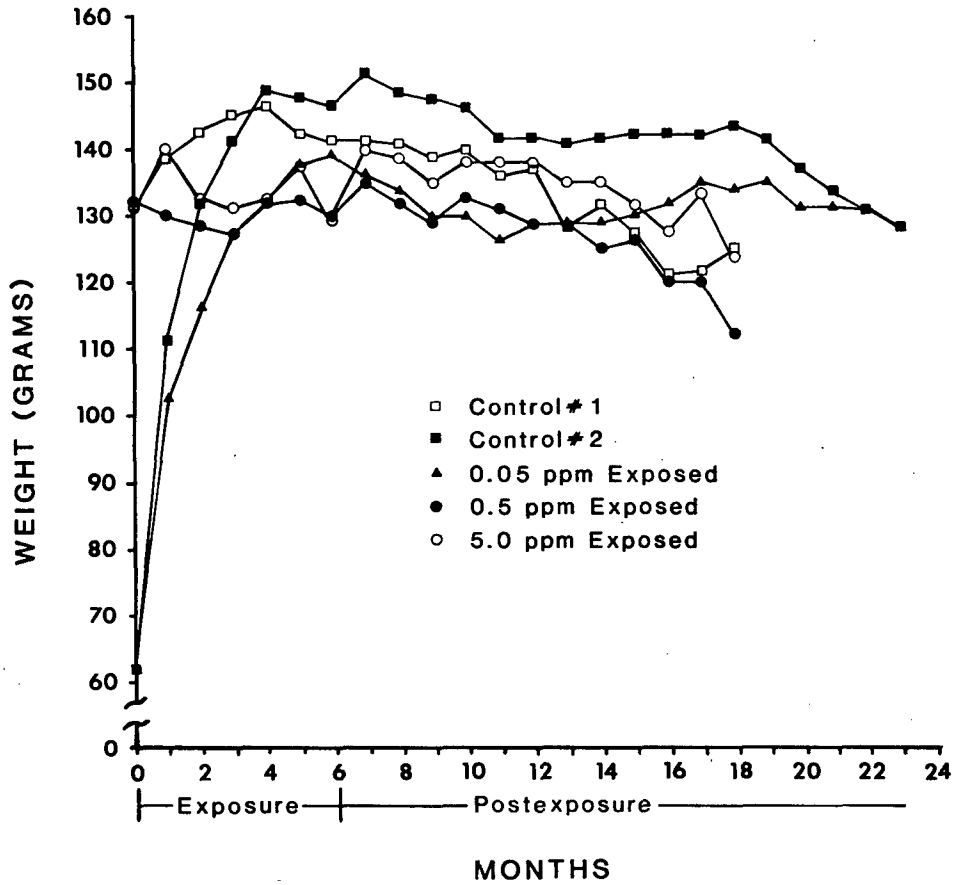

Figure 3. Effect of chronic exposure to inhaled Propellant Grade UDMH on the growth of male Golden Syrian hamsters. (Group mean weights)

Signs of Toxicity and Mortality

Overt signs of toxicity were not seen in animals exposed for 6 months to UDMH. Deterioration in appearance of the rats and mice over several months was noted during the postexposure phase of the experiment. This was attributed to the aging process and was not noticeable in hamsters.

The numbers of animals that died during the exposure phase of the study are shown in Table 2. Deaths were due in part to respiratory infections but mostly to accidental events in the exposure chambers.

TABLE 2. MORTALITY RATIOS IN GROUPS OF CONTROL AND UDMH EXPOSED ANIMALS AT 6-MONTH EXPOSURE CONCLUSION

Experimental Group	Male/Female Dogs	Male Rats	Female Mice	Male Hamsters
Control Set 1	0/8		21/400	13/200
Control Set 2	0/8	0/200	10/400	1/200
0.05 ppm exposed	0/8	4/200	23/400	16/200
0.5 ppm exposed	0/8	0/200	13/400	23/200
5.0 ppm exposed	0/8	1/200	9/400	24/200

Cumulative mortality information for hamsters throughout the entire study is shown in Figure 4. Postexposure survival was greater for the 0.05 ppm group and its control set than for the 0.5 and 5 ppm groups and their controls possibly because they were 2 1/2 months younger than the other study groups at the start of the experiments. Even five months later their mortality rate was not as high.

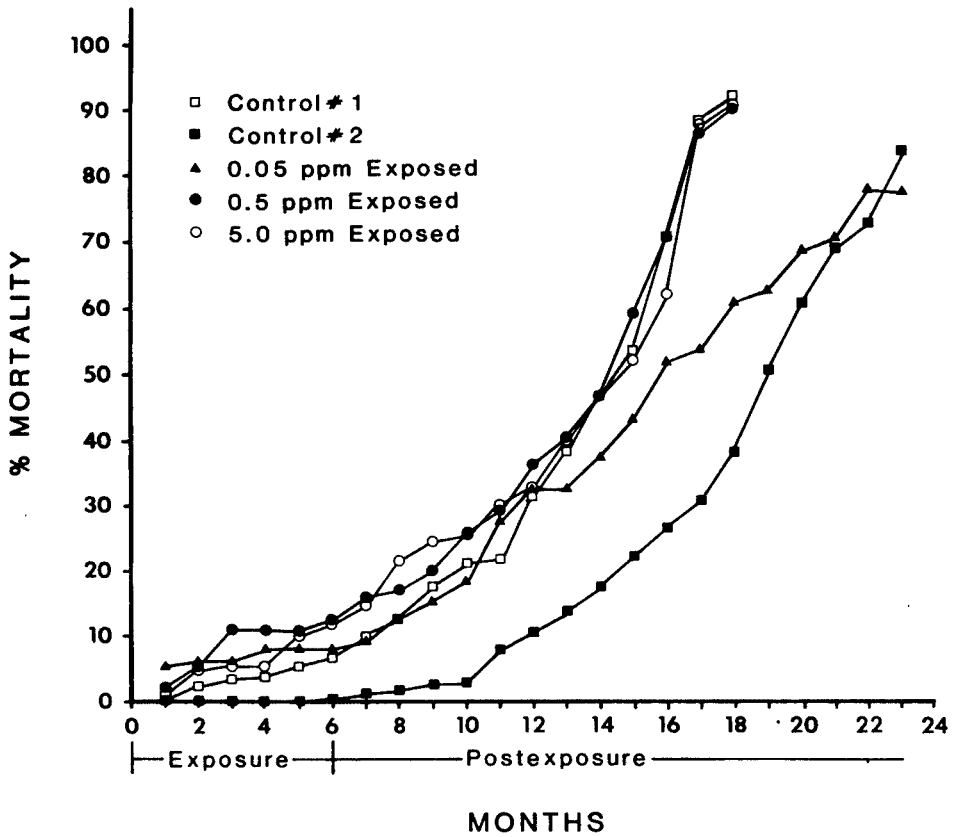

Figure 4. Cumulative mortality in Golden Syrian hamsters exposed to propellant grade UDMH and their controls. (N = 200/Group)

Mortality data for rats are shown in Figure 5. There were very few deaths during exposure and the postexposure cumulative mortality experience was comparable in all groups of rats.

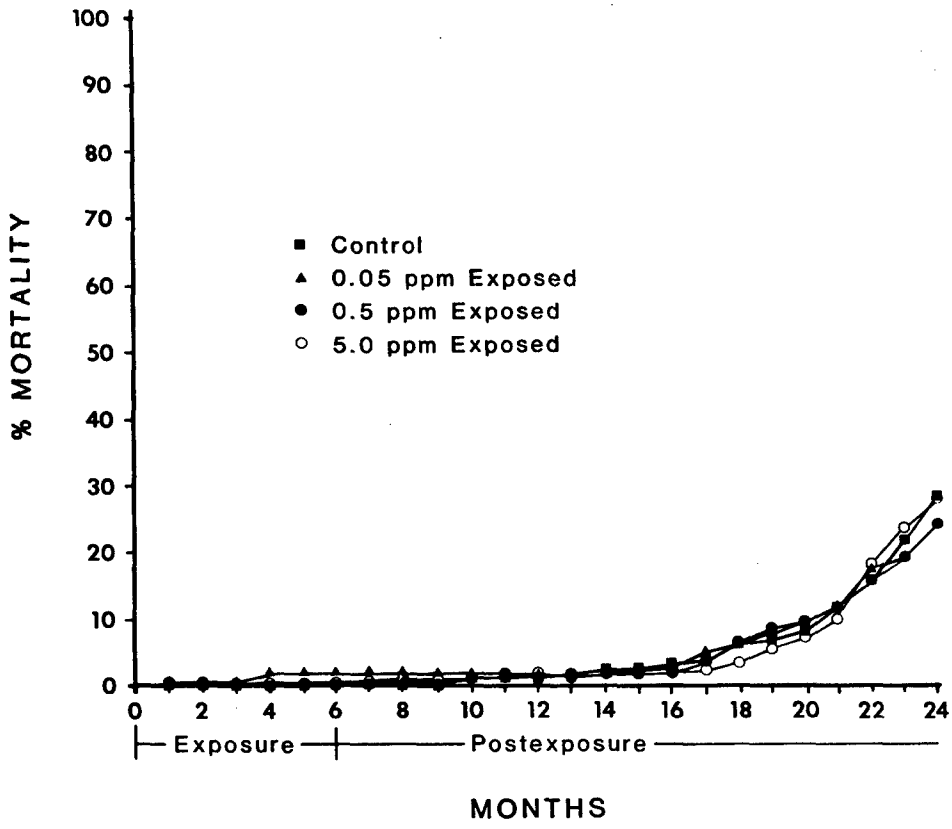

Figure 5. Cumulative mortality in male Fischer 344 rats exposed to propellant grade UDMH and their controls. (N = 200/Group)

Cumulative mortality for mice presented in Figure 6 shows no dose related increase during the 6 months of exposure to UDMH. Natural attrition due to aging in all groups is reflected in the rapid increase in mortality between 13 months postexposure and study termination.

One male dog exposed to 5 ppm UDMH died 15 months postexposure. Another male dog exposed to 0.05 ppm was euthanized 44 months postexposure due to paralysis.

Clinical Hematology and Chemistry Measurements

Significant exposure effects of inhaled UDMH were limited to transitory hepatotoxicity in dogs exposed to the 5 ppm UDMH

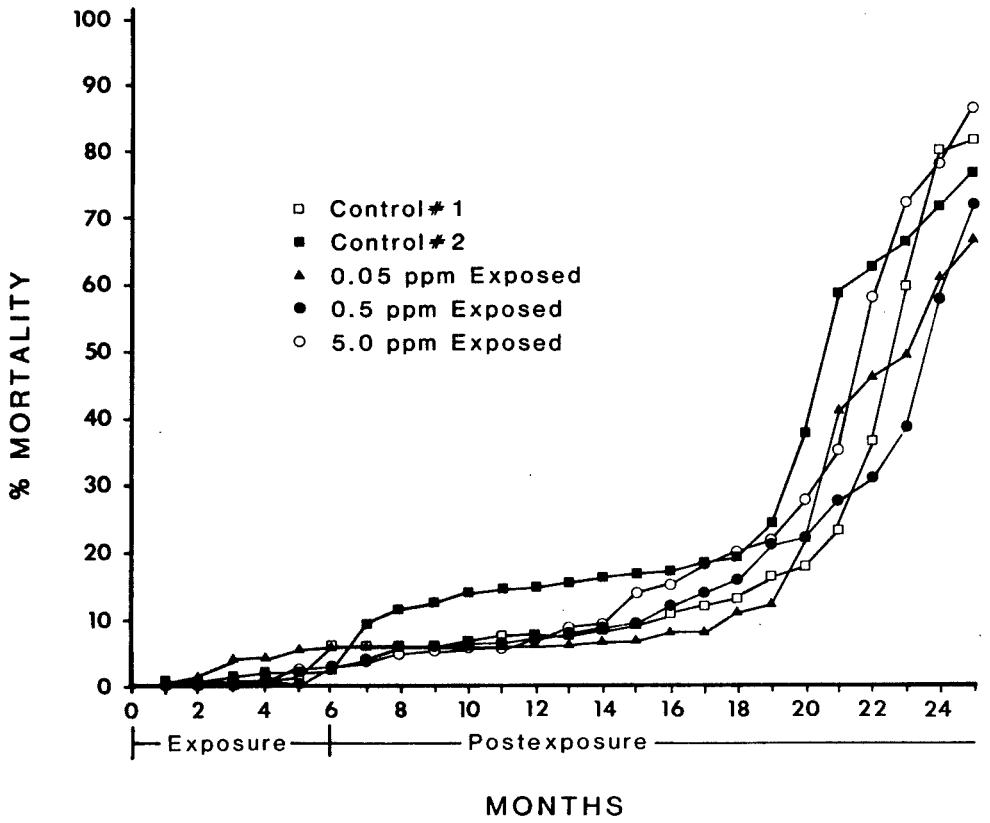

Figure 6. Cumulative mortality in female C57BL/6 mice exposed to propellant grade UDMH and their controls. (N = 400/Group)

concentration. These dogs developed elevated serum glutamic pyruvic transaminase (SGPT) levels by the fourth week of exposure and at 6 weeks the mean SGPT value was 3 times the control level. Throughout the remaining 20 weeks of exposure, SGPT values for the exposed dogs (Table 3) were stable at levels 3-4 times those of the control group. A trend to recovery, approximately 50% reduction, was seen in measurements made 2 weeks postexposure. Subsequent values at 4, 8, and 11 weeks postexposure showed no further reductions. However, when the dogs were sampled again (at Brooks Air Force Base, Texas where they were being maintained) at 27 and 47 weeks postexposure, SGPT values were not different from control animals values.

Liver function tests were performed on the 0.5 and 5 ppm exposed dogs and their controls at exposure termination. Subsequent tests were done only on the 5 ppm exposed and control dogs at 4, 8, 11, and 38 weeks postexposure, since values were normal for the 0.5 ppm dogs at exposure conclusion. Bromsulphalein (BSP) measured in the blood of the 5 ppm UDMH exposed dogs 10 minutes following a 10 mg/kg injection showed significant retention at exposure termination, 4

TABLE 3. EFFECT OF 6-MONTH INHALATION EXPOSURE TO 5.0 PPM UDMH ON SERUM GLUTAMIC PYRUVIC TRANSAMINASE LEVELS[a] IN DOGS

[Group Mean Values (N = 8)]

Weeks of Exposure	Unexposed Control Group	5.0 ppm Exposed Group
2	26	32
4	27	79[b]
6	27	102[b]
8	25	118[b]
10	26	118[b]
12	31	116[b]
14	--	--
16	22	88[b]
18	23	107[b]
20	23	99[b]
22	20	97[b]
24	22	100[b]
26	25	86[b]
Weeks Postexposure		
2	22	37[b]
4	23	42[b]
8	22	36[b]
11	23	35[b]
27	33	30
47	40	37

[a] International Units
[b] Statistically different from controls at $p \leq 0.01$

and 8 weeks postexposure (Table 4). Liver function as measured by BSP retention appeared normal at 11 weeks postexposure. BSP measurements performed 38 weeks postexposure showed no abnormal values.

Other clinical tests conducted on blood samples from dogs at the end of the 0.5 and 5 ppm UDMH exposures showed no abnormalities when compared with control values. Likewise, examination of hematocrit and red blood cell determinations made immediately postexposure on 0.5 and 5 ppm exposed rats and hamsters revealed no significant differences from control values.

TABLE 4. MEAN BROMSULPHALEIN RETENTION VALUES[a] IN CONTROL AND 5.0 PPM UDMH EXPOSED DOGS

Time (Weeks)	Control	5.0 ppm
Exposure Termination		
26	18.1	30.3[b]
Postexposure		
4	20.7	29.5[b]
8	12.8	30.0[b]
11	18.0	21.8
38	11.4	12.3

[a] Percent retention at 10 minutes
[b] Statistically different from controls at $p \leq 0.05$

Pathology

Gross and histopathologic examinations were conducted on all animals that died or were sacrificed during and at the completion of the study. Autolysis or cannibalization prevented complete examination in some cases. As noted earlier, one group of unexposed control rats was affected by an infectious agent which rendered the survivors unsuitable for use as a control group. In the case of mice, the growth and mortality patterns of both sets of unexposed controls were similar enough that they were combined for comparisons of incidence of pathologic lesions between controls and UDMH exposed groups.

Mice

Table 5 shows tumor incidence in the various groups of exposed and control mice. The outstanding finding in mice was the dose related increase in hemangiosarcomas, thyroid carcinomas, and Kupffer cell sarcomas. In the 5 ppm exposure group, there were 17/360 hemangiosarcomas versus 5/701 in the control group. Also eight Kupffer cell sarcomas were seen compared with one in the control group. The numbers are modest, but significant in the 5 ppm group. There is a fairly large incidence of malignant lymphomas in each group of exposed and control mice. This is a common finding in aging C57BL/6 mice, but the incidence in the 5 ppm group was statistically higher than the incidence in their controls. The number of alveolar bronchiolar adenomas in the 5 ppm group was also larger than the number seen in its controls, but without statistical significance.

TABLE 5. SELECTED[a] TUMORS FOUND IN FEMALE C57BL/6 MICE AFTER INHALATION EXPOSURE TO PROPELLANT GRADE UDMH[b]

Tumor type	Unexposed Controls	Exposed 0.05 ppm UDMH	Exposed 0.5 ppm UDMH	Exposed 5.0 ppm UDMH
Lung				
Adenoma[c]	9/660	4/355	7/331	11/337
Carcinoma	1/660	1/355	3/331	0/337
Liver				
Hepatocellular Carcinoma	4/681	1/363	7/344	4/342
Pituitary				
Adenoma[d]	91/525	44/320[e]	26/257[e]	27/251[e]
Carcinoma	5/525	3/320	2/257	1/251
Thyroid Follicular Cell				
Adenoma	45/551	17/311	20/278	13/286
Carcinoma[d]	2/551	1/311	8/278[e]	5/286[e]
Uterus				
Leiomyosarcoma	1/632	3/348	3/311	3/312
Ciculatory System				
Hemangioma	7/701	2/374	2/368	5/360
Hemangiosarcoma[d]	5/701	9/374[e]	3/368	17/360[e]
Hematopoietic System				
Malignant Lymphomas	181/701	102/374	98/368	112/360
Kupffer Cell Sarcoma[d]	1/701	4/374[e]	0/368	8/360[e]
Plasma Cell Tumors	2/701	4/374	1/368	2/360

[a] Selection based on frequency of appearence and those possibly exposure related
[b] Number of mice with tumors/Number examined
[c] Linear trend significant at 0.05 level
[d] Linear trend significant at 0.01 level
[e] Significantly different from control at 0.01 level

Frequently seen non-neoplastic pathologic changes in mice are listed in Table 6, some of which appear to be UDMH exposure related. Although nasal cavity tissue was examined, the results are

TABLE 6. SELECTED[a] NON-NEOPLASTIC LESIONS IN FEMALE C57BL/6 MICE AFTER INHALATION EXPOSURE TO PROPELLANT GRADE UDMH[b]

Lesion type	Unexposed Controls	Exposed 0.05 ppm UDMH	Exposed 0.5 ppm UDMH	Exposed 5.0 ppm UDMH
Lung				
Congestion[c]	179/686	83/340	121/331[d]	98/336[e]
Perivascular Cuffing[f]	121/686	54/340	93/331[d]	90/336[d]
Lymphoid Hyperplasia[f]	4/686	6/340[d]	14/331[d]	8/336[d]
Liver				
Congestion	48/681	20/363	47/344[d]	20/342
Angiectasis[f]	11/681	9/363[e]	2/344[d]	23/342[d]
Gallbladder				
Hyaline Degeneration[f]	48/701	49/374[d]	45/368[d]	41/360[d]
Eosinophilic Cytoplasmic Change	23/701	21/374	14/368	20/360
Ovary				
Cysts	98/603	55/336	44/287	67/308
Uterus				
Endometrial Cysts[f]	11/632	46/348[d]	67/311[d]	57/312[d]

[a] Selection based on frequency of appearance and those possibly exposure related
[b] Number of mice with lesions/Number examined
[c] Linear trend significant at 0.05 level
[d] Significantly different from control at 0.01 level
[e] Significantly different from control at 0.05 level
[f] Linear trend significant at 0.01 level

not shown since lesions were absent or of a very low incidence in either exposed and control animals. Lung congestion, a common post mortem finding, was seen more frequently in the 0.5 ppm exposed mice than in their control group. Pulmonary perivascular cuffing and lymphoid hyperplasia may be part of generalized lymphomas or simply represent mild inflammatory changes as the result of aging. The incidence is greater in all UDMH exposed groups than in controls.

The examination of liver tissue revealed a large number of lesions in all groups. Angiectasis in the 5 ppm exposed group was the only finding viewed as exposure related. Sometimes the pathologist had difficulty in distinguishing simple angiectasis from true neoplastic transformation of endothelial cells in the form of hemangiomas/hemangiosarcomas. Hyaline degeneration of the gallbladder occurred more frequently in all exposed groups than in controls. Since UDMH is metabolized and excreted by the liver, these findings are possibly indicative of UDMH toxicity. Ovarian and uterine cysts are extremely common in aged female mice and are probably not related to UDMH exposure but it is noteworthy that the number of uterine cysts seen in all groups of exposed mice is considerably greater than the number seen in control groups.

Rats

Selected pathologic lesions seen in the rats are shown in Table 7. Two types of lung tumors are listed. Bronchiolar adenomas are somewhat more prevalent with a statistically significant increase in the high dose group. A significant increase in pancreatic islet cell adenomas is seen in the 0.5 ppm dose group. The high UDMH exposure group does show a higher incidence than controls, but it is not statistically significant. Chromophobe adenomas are prevalent in aged Fischer 344 rats and are seen in a higher incidence in all exposed groups. Fibrous histiocytomas are a fairly common tumor seen in the skin and soft tissue of rats. There were increases of this type seen in both the low and intermediate dose groups, but not in the highest exposure group.

Inflammatory lesions of the respiratory system were frequent findings in all groups of exposed and control rats and were part of the complex of chronic murine pneumonia commonly seen in rats. Alveolar epithelial hyperplasia includes findings described as adenomatous hyperplasia, adenomatosis, bronchiolar epithelial hyperplasia, bronchiolar papillary hyperplasia, fibrosis with epithelialization, and hyperplasia of goblet cells. Although the frequency of hyperplasia is low in all groups, the incidence in all exposed groups is statistically greater than in the control groups. Alveolar hyperplasia is an important finding in that it can represent early neoplastic lesions. Subsequent manifestations of this process may be the bronchiolar adenomas seen in the 5 ppm exposed rats.

TABLE 7. SELECTED[a] PATHOLOGIC LESIONS FOUND IN MALE FISCHER 344 RATS AFTER INHALATION EXPOSURE TO PROPELLANT GRANDE UDMH[b]

Lesion type	Unexposed Controls	Exposed 0.05 ppm UDMH	Exposed 0.5 ppm UDMH	Exposed 5.0 ppm UDMH
Lung				
Bronchiolar Adenoma	5/189	0/192	2/182	10/191[c]
Squamous cell Carcinoma	1/189	1/192	1/182	2/191
Alveolar hyperplasia	8/189	17/192	16/182	11/191
Liver				
Hepatocellular adenoma	4/197	7/193	1/189	3/188
carcinoma	2/197	0/193	2/189	3/188
Bile duct hyperplasia	146/197	130/193	153/189	153/188
Cytoplasmic vacuolization	5/197	10/193	29/189	7/188
Fatty change[d]	59/197	69/193[c]	38/189[c]	38/188[c]
Pancreas				
Islet cell Adenoma[d]	0/170	3/174[c]	12/169[c]	6/158[c]
Carcinoma	0/170	1/174	0/169	2/158
Pituitary				
Chromophobe adenoma[d]	60/171	76/182[c]	75/169[c]	90/174[c]
Kidney				
Carcinoma	0/195	1/196	0/196	1/190
Adrenal				
Adenoma	2/194	2/188	1/177	3/188
Carcinoma	1/194	0/188	0/177	0/188
Miscellanous				
Mononuclear cell leukemia[d]	67/200	40/197[c]	19/193[c]	37/196[c]
Fibrous Histiocytoma	0/200	4/197	4/193	0/196

[a] Selection based on frequency of occurrence and those possibly exposure related
[b] Number of rats with lesions/Number examined
[c] Significantly different from control at 0.01 level
[d] Linear trend significant at 0.05 level

Hamsters

Lesions seen most frequently in hamsters were: adrenal cortical adenoma, reticuloendothelioma, tracheitis, adenomatosis, interstitial pneumonitis, bronchiolar hyperplasia, amyloidosis, hemosiderosis, bile duct hyperplasia, cholangitis, biliary cysts, mineralization in the kidneys, adrenal cortical hyperplasia, aspermatogenesis, and hypospermatogenesis. These lesions all reflect the aging process and disease states to which hamsters are susceptible. In no case was there a statistically significant difference in lesion incidence between exposed and control animals.

Dogs

One dog exposed to 5 ppm UDMH died 15 months postexposure, and one dog exposed to 0.05 ppm was sacrificed 44 months postexposure.

Gross findings at necropsy of the 5 ppm UDMH exposed dog revealed a large white neoplastic mass which encapsulated the heart and portions of the lung with invasion of these tissues and the costal pleura. Histologic examination revealed a reticulum cell sarcoma of multicentric origin. The sarcoma was metastatic to the lung, pleura, and vascular adventitia of thoracic vessels. The right popliteal lymph node also contained an area of reticulum cell sarcoma.

The other dog, in the 0.05 ppm exposure group, was euthanized because of posterior paresis. A ruptured thoracic intervertebral disc was found during necropsy. No neoplasms were seen in this dog.

The surviving 38 dogs were sacrificed at 51-54 months postexposure. There were no significant dose related lesions in exposed dogs. The pathologic changes seen were all non-neoplastic lesions compatible with aging changes in dogs. Most lesions involved the endocrine organs and except for one male dog, the changes were limited to female dogs.

SECTION III

COMPARATIVE HEPATOTOXIC RESPONSE OF DOGS TO THE INHALATION OF PURE UDMH AND UDMH CONTAINING DMNA

Dimethylnitrosamine (DMNA), a well-known carcinogen and hepatotoxin, was found to be present at a 0.12% concentration in the supply of UDMH used in the 6-month oncogenic study. At the time the

UDMH experiments were initiated, we were not concerned with this trace amount of DMNA as a contaminant since it was ubiquitous in any sample of UDMH. We had calculated that this amount of contamination of the UDMH would, at worst, result in a 6 ppb concentration of DMNA in the highest UDMH exposure concentration and did not think that this level of DMNA could cause any effect. However, we became concerned with the possible effect of DMNA on the experimental results of the chronic UDMH study when the dogs showed evidence of mild hepatotoxicity. Therefore, in order to obtain information on the possible hepatotoxicity of DMNA at low doses, a series of short-term inhalation experiments were conducted using beagle dogs. Additionally, as a companion study, mice were given oral doses of DMNA equivalent to various calculated inhalation concentrations to determine hepatotoxicity.

MATERIALS AND METHODS

Two male and two female beagle dogs served as test subjects and were exposed in a 2 m^3 Rochester Chamber. Two male and two female beagles served as controls and were exposed to air only in an adjacent Rochester Chamber. Both chambers were operated with nominal airflows of 30 CFM at a slightly reduced pressure to prevent leakage of the test chemical into the laboratory atmosphere. The analysis of chamber atmosphere was continuous, utilizing the AutoAnalyzer colorimetric technique used for monitoring UDMH in the 6-month inhalation study. Blood samples were drawn from all dogs at bi-weekly intervals or more often and clinical determinations made for the following battery of tests:

RBC	Calcium
WBC	Glucose
HCT	Total Protein
HGB	Albumin
Differential Cell Count	Globulin
Sodium	SGPT
Potassium	Alkaline Phosphatase

Bromsulphalein (BSP) retention times were determined at the beginning and end of the study. Baseline measurements were available for all dogs for at least 2 months prior to this study, thus insuring selection of healthy animals with stable blood parameters.

In the first and second experiments of the series, the dogs were exposed to 5 ppm UDMH determined to be free of DMNA by mass spectrometric analysis. In the final test, the dogs were exposed to a mixture of 0.12% DMNA in UDMH which gave an air concentration of an estimated 6.0 ppb DMNA and essentially 5 ppm measured UDMH.

The first exposure was conducted for 8 1/2 weeks on a 6 hour/day schedule omitting weekends and holidays. This was a duplication of the exposure regimen used in the 6-month study. To examine the possibility that 5 ppm UDMH (free of DMNA) may have caused liver changes not revealed by SGPT measurements, liver wedge biopsies were taken from all exposed and control dogs for pathologic examination at exposure conclusion.

The dogs were rested 5 days following biopsy surgery after which the test dogs were continuously exposed to 5 ppm UDMH (free of DMNA) and the control dogs to air only for 13 days to see whether this continuous challenge would cause SGPT changes in exposed animals.

In a final test of this series, the female control dogs were placed in the exposure chamber and the female dogs previously exposed to the purified UDMH were placed in the control chamber. This interchange was made to take into account the possibility of sensitization of the dogs by exposure to UDMH in the first two experiments. The male dogs were not interchanged. The experiment was started within a few hours after the cessation of the previous one and the dogs were exposed for 16 days continuously to air or the mixture of UDMH and DMNA. Following exposure, the study was concluded and all dogs were killed and submitted for gross and histopathologic examination.

RESULTS

There were no significant changes in mean SGPT values for the exposed dogs as a result of 8 1/2 weeks of intermittent exposure followed by 13 days continuous exposure to 5 ppm purified UDMH (Table 8). All other clinical chemistry determinations were normal when compared to control values. The results of histopathologic examination of liver wedge biopsies taken from all exposed and control dogs at the conclusion of 8 1/2 weeks of exposure showed some marginal differences between exposed and control tissues. Cytoplasmic degenerative change in liver cord cells was greater and occurred more frequently in exposed animals. There was also a modest increase in the amount of yellow-brown granular hemosiderin material accumulated in Kupffer cells of exposed dogs.

There were significant SGPT elevations (Table 9) in blood samples taken from dogs exposed continuously to 5 ppm UDMH containing 0.12% added DMNA for 16-days. Noticeable is the 25% increase in mean SGPT values from 10 through 16 days of exposure. BSP determinations made on all dogs at exposure termination showed no significant differences between exposed and control mean values, nor any trend toward elevations in individual values for exposed dogs.

TABLE 8. MEAN SGPT VALUES OF DOGS EXPOSED TO 5.0 PPM PURIFIED UDMH[a] AND SHAM EXPOSED CONTROLS

Sample Period	Mean SGPT Value (International Units) Exposed[b]	Control[b]
Preexposure		
2 Months	36.8	30.0
1 Month	25.8	24.0
1 Week	31.5	30.0
Intermittent Exposure		
2 Weeks	37.8	32.8
4 Weeks	37.8	34.0
6 Weeks	32.0	29.0
8 1/2 Weeks	36.8	32.0
Before Continuous Exposure	48.9	45.1
7 Day Continuous Exposure	41.5	37.5
13 Day Continuous Exposure	44.8	30.1

[a] Free of DMNA by mass spectrometric analysis
[b] N = 4

TABLE 9. MEAN SGPT VALUES OF DOGS AFTER CONTINUOUS EXPOSURE TO 5.0 PPM PURIFIED UDMH CONTAINING 0.12% ADDED DMNA[a]

Sample Period	Mean SGPT Value (International Units) Exposed[b]	Sham Control[b]
Days of Exposure		
10	68.8[c]	42.3
16	85.8[c]	37.5

[a] Determined by mass spectrometric analysis
[b] N = 4
[c] Statistically different from controls at $p \leq 0.05$

All other clinical chemistry determinations were normal when compared to control values. Gross pathology examination revealed nothing more than small lesions resulting from the liver biopsies taken after 8 1/2 weeks of intermittent exposure to the purified UDMH. Histopathology

results were the same for exposed and control dogs. Hepatocytes were relatively uniformly pale and swollen although this alteration was slightly more prominent in the periacinar regions. The cytoplasm contained many eosinophilic granules as well as some yellow-brown pigment granule. The latter were also noted in Kupffer cells. Several nonspecific eosinophilic intranuclear inclusions were also seen.

SECTION IV

PERORAL TOXICITY OF DIMETHYLNITROSAMINE IN MICE

As a companion experiment to the study of the hepatotoxic response of dogs to purified UDMH and a mixture of purified UDMH with DMNA, mice were given oral doses of DMNA to evaluate the hepatotoxicity in this species.

MATERIALS AND METHODS

The CF-1 male mice used in this study, obtained from Harlan Industries, had a weight range of 20-25 grams. The DMNA supply, +99% purity, was purchased from Aldrich Chemical Company. Glass syringes with special dosing needles were used to intragastrically administer the various solutions of DMNA in distilled water. The non-fasted mice were weighed prior to dosing to determine the desired injection volume. Control mice received a daily peroral treatment with distilled water.

The mice were given repeated oral doses of DMNA each working day over a period of 28 calendar days. Mice at the two lowest concentrations and the controls continued to be dosed through 60 calendar days. The lowest dose given was the oral equivalent of the calculated inhalation concentration of DMNA expected to result from 6-hour exposures to 5.0 ppm propellant grade UDMH. Calculations were based on a mouse weight of 25 grams and minute volume of 23 ml/min. The oral doses used in this study along with the equivalent inhalation concentrations are listed in Table 10.

Five mice from each group (including controls) were sacrificed after 7, 9, 14, 21, and 28 calendar days. At the four highest dose levels, all survivors were sacrificed after 28 days. Animals that received the two lowest dose levels and the controls continued to receive daily doses through 60 calendar days at which time all survivors were sacrificed.

TABLE 10. COMPARISON OF DAILY ORAL DOSES OF DMNA GIVEN TO MICE WITH THE CALCULATED THEORETICAL EQUIVALENT 6-HOUR INHALATION CONCENTRATION

Oral Dose	Inhalation Equivalent[a]	Mice per Group
5 µg/kg	5 ppb	55
10 µg/kg	10 ppb	55
50 µg/kg	50 ppb	35
500 µg/kg	500 ppb	35
1 mg/kg	1000 ppb	35
5 mg/kg	5000 ppb	35
Controls	0 ppb	55

[a] Calculated for a 6-hour exposure

The livers were removed from any mice that died or were sacrificed. The liver tissue was processed and prepared for histopathologic examination which included electron microscopic analysis.

RESULTS

Very few deaths occurred during the dosing regimen. Most deaths were caused by extraneous infections and were unrelated to the toxic action of the contaminant or trauma induced by the dosing procedure.

Pathology

Light Microscopy

Histopathologic examination of the mice sacrificed at the prescribed time periods revealed some fatty change in the liver of mice receiving doses of 50 µg/kg DMNA or higher. A summary of the histologic findings is given in Table 11. Mice from the two lowest dose levels showed varying degrees of hepatocyte vacuolation in 73 to 79% of the mice examined. However, the control group shows similar effects in 64% of the mice which indicates that the small increase in the two low dose groups over the control incidence may be insignificant. The incidence of hepatocyte vacuolation in all other dose groups of 90% or greater is biologically significant. The liver of the mice at the highest UDMH dose level (5 mg/kg) also had a high incidence (94%) of hepatocellular necrosis, a definite indication of liver tissue damage.

TABLE 11. LIVER CHANGES IN MICE AFTER REPEATED ORAL EXPOSURE TO DIMETHYLNITROSAMINE

DMNA Concentration	Effects Observed
Controls	Hepatocyte vacuolation in 64% of mice.
5 µg/kg	Hepatocyte vacuolation in 79% of mice.
10 µg/kg	Hepatocyte vacuolation in 73% of mice.
50 µg/kg	Hepatocyte vacuolation in 90% of mice.
500 µg/kg	Hepatocyte vacuolation in 92% of mice.
1 mg/kg	Hepatocyte vacuolation in 97% of mice.
5 mg/kg	Hepatocyte vacuolation in 97% of mice. Hepatocellular necrosis in 94% of mice.

Electron Microscopy

Livers of mice administered the two lowest dose levels of DMNA were all similar in appearance at all examination times. Some lipid droplets were seen within the hepatocyte cytoplasm, as were a few autophagic vacuoles per cell. Additionally, myelin figures were noted in the cells of the dosed animals.

The hepatocytes in livers from mice dosed at the 50 µg/kg level were similar to the lower dosed groups and controls at 7 and 9 days. At 14 days the cytoplasm of some cells appeared very electron lucent and after 21 days the rough endoplasmic reticulum (RER) of some cells appeared dilated.

In mice treated with progressivly higher doses of DMNA, the time to appearance of hepatocyte changes was decreased. In both the 1 mg/kg and 5 mg/kg dose groups these changes were seen in the first set examined after 5 treatments. There was a progressive increase of autophagic vacuoles which later became filled with dense osmophilic debris. These were seen in hepatocytes near the central hepatic vein. At the highest dose level many mice had markedly increased electron lucency to the cytoplasm of their hepatocytes, some of which contained cellular organelles and degenerative changes corresponding to the necrotic changes seen with light microscopy.

UCI Exp # 457-458

SECTION V

A STUDY OF THE ONCOGENIC POTENTIAL OF INHALED PURIFIED UDMH IN MICE

Results of histologic evaluation of the tissue of rodents exposed to UDMH in the 6-month chronic exposure study indicated that mice were the most susceptible species tested, with significant increases in circulatory and liver tumors in exposed animals.

The tumor incidence in mice chronically exposed to 5 ppm UDMH could not be conclusively associated with UDMH since there was simultaneous exposure to 0.12% (6 ppb) DMNA, a potent liver toxin. The DMNA, rather than UDMH, could have been the responsible agent for the tumors. Evidence that this very small amount of DMNA does cause hepatotoxicity in dogs and hepatocyte changes in mice left some uncertainty as to the cause of the increased tumor incidence seen in the first study. In an attempt to resolve the uncertainty in interpretation of the chronic inhalation exposure results, a second inhalation study was conducted with mice exposed to purified UDMH free of DMNA.

MATERIALS AND METHODS

Animals

Female C57BL/6 mice purchased from Charles River Breeding Laboratories were used for the inhalation study. Commercial chow was made available during nonexposure hours and was removed during exposure to prevent absorption of UDMH on the food and subsequent injestion by the mice. Water was available ad libitum. The mice were approximately 9 weeks of age at the beginning of the study.

Experimental Design

Two hundred female mice were exposed for one year to 5 ppm purified UDMH vapor in Rochester Chambers of 2 m^3 volume. Two chambers were used for the exposures with each chamber containing 100 mice. An equal number of female mice serving as sham exposed controls were maintained in two Longley chambers 2.5 m^3 in volume. During the exposure period the mice were housed in stainless steel wire cages with 25 mice per cage. Environmental operating conditions for the chamber were identical through the course of the study with a common air supply line serving all four chambers. The exposures were conducted following an industrial work week type schedule of 5 days/week, 6 hours/day with no exposures holidays. The exposure

regimen was designed to simulate human exposure situations and parallel the conditions of the previous chronic UDMH inhalation exposure.

Body weights were taken monthly during the entire course of the study. Weights were obtained by cage group. At the conclusion of the exposure, all mice were removed from the chambers and housed in laminar air flow facilities for one year of postexposure observation. At the end of the observation period, all remaining mice were sacrificed. The tissues from these mice as well as the tissues from mice dying during the exposure period were taken for histologic examination. Approximately 33 tissues were sampled in each animal using the National Cancer Institute protocol. The tissues taken were the same ones as were sampled in the chronic study, except for the deletion of male reproductive organs since only female animals were used in the current study.

Two extra mice were included in the exposed and control groups for electron microscopic examination midway through exposure. These mice were removed after six-month's exposure according to plan. Lungs were collected from each animal, and the bronchi, respiratory bronchioles, and alveoli were examined using scanning electron microscope techniques. Livers were also collected and examined using transmission electron microscopy.

Chemical Analysis and Generation

The UDMH used in this experiment was redistilled from rocket propellant grade UDMH to remove the dimethylnitrosamine. The redistilled UDMH was stored in teflon-lined, capped, brown 100 mL glass bottles under nitrogen. This storage method had been shown to prevent oxidative degradation of monomethylhydrazine for periods greater than one year.

Each bottle of UDMH was analyzed for DMNA content before and during use with a gas chromatographic method developed in our laboratory. The lower limit of detection for DMNA in UDMH by this method was 7 µl/L of DMNA. Since no DMNA was detected in the bottles of UDMH used in this study, the upper limit of DMNA that could have been present in the chamber was approximately 35 parts per trillion, at least 2 orders of magnitude lower than occurred in the previous study.

A Sage Model 355 syringe pump with a 10 mL glass syringe in a contaminant introduction hood was used for chamber UDMH introduction; one for each chamber. The UDMH was evaporated in a 500 mL/minute sample air stream without additional heat and then introduced in

the chamber air supply line where the air flow quantity was maintained at 20 CFM. Reaction of UDMH with chamber ducts and walls resulted in a loss of about 10% at equilibrium.

The chamber UDMH concentrations were sampled just above the breathing zone of the animals. The sample was pulled through polyethylene tubes to an electric two-way valve which sampled for 10 minutes from one chamber and then 10 minutes from the other. The samples were analyzed with an MDA Model 7020 hydrazine analyzer using a sample flow of 31 mL/minute. The switch and analyzer were mounted between the chambers to reduce the distance of sample travel. This was necessary with low sample flow to reduce the equilibration time. Each MDA tape was calibrated before use and at least one calibration point was run each week thereafter using gas bag standards containing known amounts of UDMH.

RESULTS

Concentration Measurements

Good control was maintained on the desired 5 ppm UDMH concentration in both chambers with little variation between the chambers. The overall mean for each chamber was within 0.5% of the desired 5 ppm UDMH concentration. Monthly mean UDMH concentrations through the 12-month exposure period are shown in Table 12.

TABLE 12. MONTHLY MEAN PURIFIED UDMH CONCENTRATIONS (PPM) THROUGH THE 12-MONTH EXPOSURE PERIOD

Month	Chamber A	Chamber B
1	5.00	4.96
2	5.00	4.98
3	4.99	5.00
4	5.00	5.04
5	5.00	4.94
6	4.98	4.99
7	4.99	4.99
8	5.02	4.98
9	4.93	4.91
10	4.99	5.02
11	5.01	5.06
12	5.05	5.00
$\bar{x}$	4.99	4.98
S.D.	0.03	0.04

Body Weights

The effect of UDMH exposure on mouse body weight is shown in Figure 7. Differences in mean body weights between 5 ppm UDMH exposed mice and unexposed control mice were evident at 7 months of exposure. These differences continued through the one-year post-exposure observation period. At the conclusion of the study, a 15% difference in body weight between the control group and the exposure group had occurred.

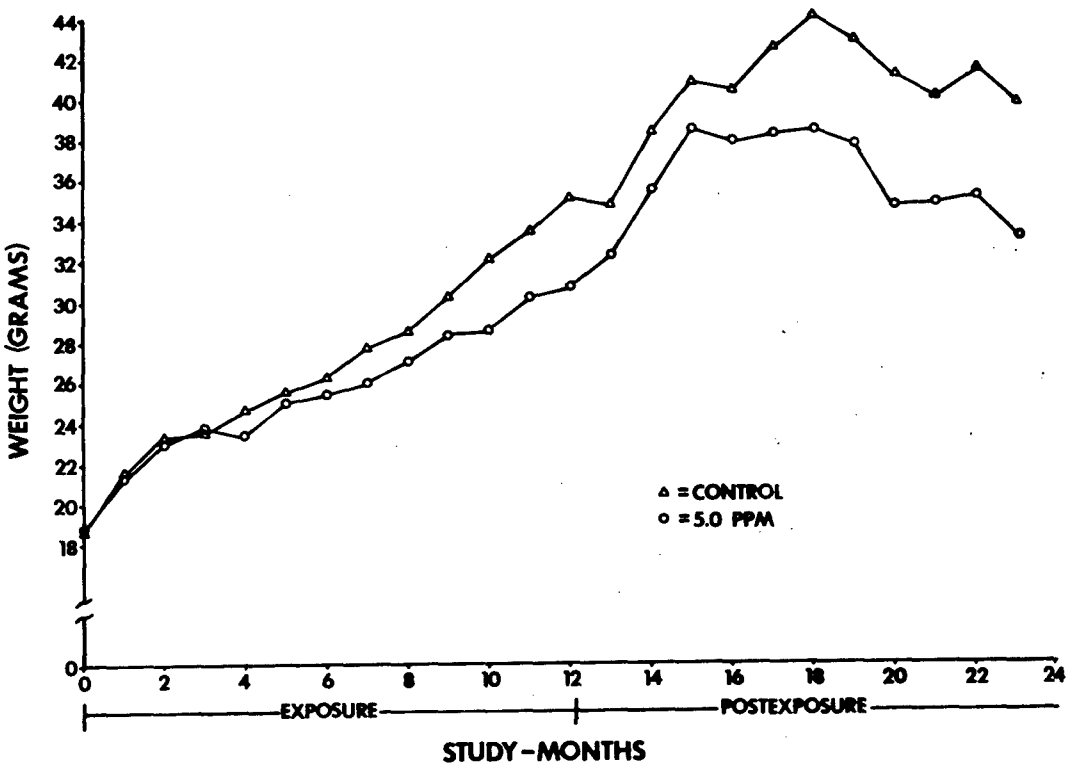

Figure 7. Effect of inhaled purified UDMH on female mouse body weight.

Mortality

The effect of exposure to 5 ppm purified UDMH vapor on survival of the mice is shown in Figure 8. At the conclusion of the one-year exposure approximately 90% of the mice in both groups were alive. Although mortality during exposure was slightly greater in UDMH exposed mice, statistical analysis of exposed and control mortality ratios showed no significant differences. The survival rate during subsequent postexposure months was slightly greater in the UDMH exposed group when compared to the unexposed control group. However, at the conclusion of the study the numbers of surviving animals in either group were essentially equal.

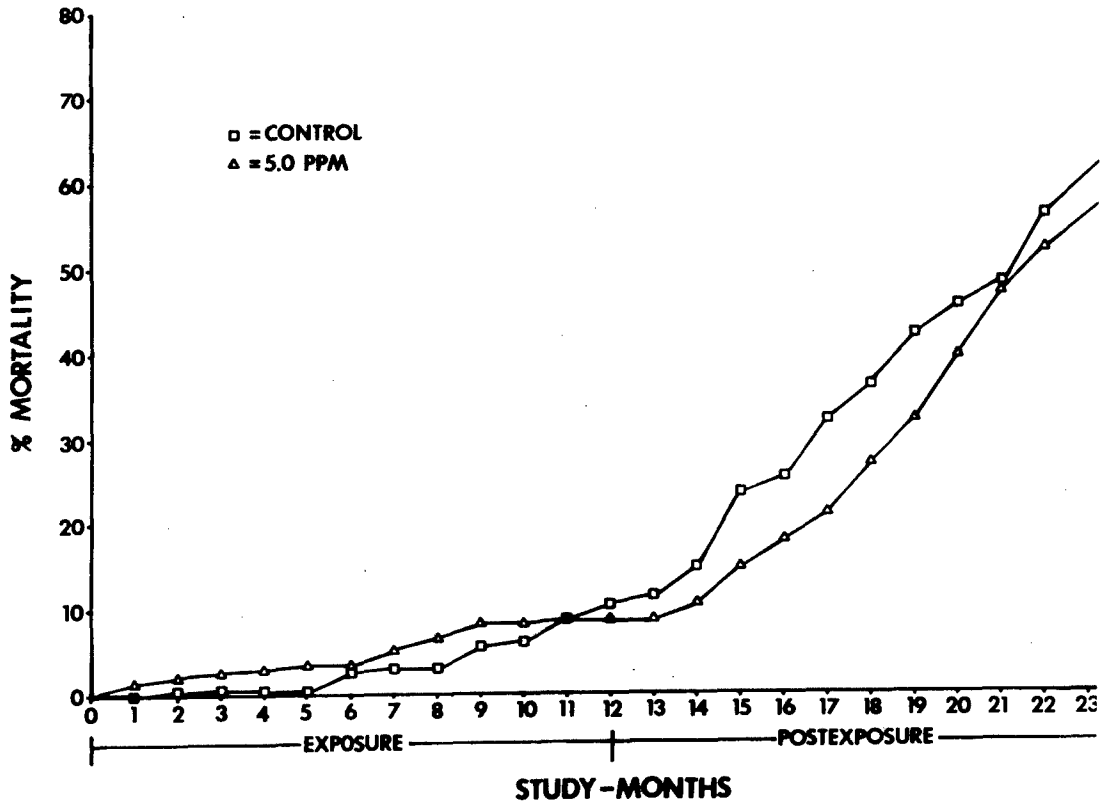

Figure 8. Mortality of female mice exposed to purified UDMH for one-year.

Pathology

Electron microscopic examination of the lungs and livers of two mice exposed to 5 ppm purified UDMH after 6 months of the one year exposure showed these tissues to be morphologically normal and no different from the lungs and livers of two control mice.

Light microscopic examination of tissues of animals that died during the study or at termination were conducted and the significance of differences in lesion incidence between test and control groups was evaluated using the Fisher Exact Test.

Most non-neoplastic lesions seen were the result of normal aging processes and occurred equally in the UDMH exposed and control group. However, there were four body sites where increases in various non-neoplastic lesions were noted in the exposed animals. These areas and lesion incidence are shown in Table 13. The nasal mucosa of exposed mice showed a number of signs of irritation. Suppurative

TABLE 13. SELECTED[a] NON-NEOPLASTIC LESIONS IN C57BL/6 MICE AFTER INHALATION EXPOSURE TO PURIFIED UDMH[b]

	Unexposed Control	5.0 ppm Exposed
Nasal Mucosa		
Inflammation, Suppurative	8/183	31/179[c]
Hyperplasia	1/183	7/179[d]
Metaplasia, Squamous	1/183	19/179[c]
Dysplasia	2/183	14/179[c]
Circulatory System		
Angiectasis	27/191	54/190[c]
Anus		
Prolapse	2/92	13/93[c]
Erosion	13/92	30/93[c]
Hyperkeratosis	12/92	16/93
Reproductive		
Endometrial Gland Cyst	0/187	6/169
Ovary Cyst	26/168	31/151
Gall Bladder		
Hyaline Degeneration	9/158	14/156

[a] Selection based on frequency of occurrence and those possibly exposure related
[b] Number of mice with lesions/Number examined
[c] Statistically higher than controls, $p \leq 0.01$
[d] Statistically higher than controls, $p \leq 0.05$

inflammation and squamous cell metaplasia were the most frequently observed lesions in the nasal tissue of the exposed mice. Abnormally dilated blood vessels (angiectasis) was a prominent finding in exposed animals at an incidence rate twice as much as control animals. Although these lesions were found in other organs, the highest incidence was in the liver (6/191 control and 17/190 exposed). Non-neoplastic lesions of the anal and reproductive tissues were also observed more frequently in the UDMH exposed mice than in the control group.

There were multiple sites of tumor formation observed in the UDMH exposed mice: lung, liver, nasal cavity, circulatory system, and lymphatic system. As shown in Table 14, tumors were also seen in control animals at all sites except for the nasal cavity.

TABLE 14. SELECTED[a] TUMORS FOUND IN C57BL/6 MICE AFTER INHALATION EXPOSURE TO PURIFIED UDMH[b]

	Unexposed Control	5.0 ppm Exposed
Lung		
Alveolar/Bronchiolar Adenoma	4/187	20/186[c]
Alveolar/Bronchiolar Carcinoma	0/187	3/186
Liver		
Hepatocellular Adenoma	4/187	20/188[d]
Hepatocellular Carcinoma	0/187	1/188
Lymphatic System		
Malignant Lymphomas (All Types)	64/191	84/190[d]
Nose		
Papilloma	0/183	5/179[c]
Papillary Carcinoma	0/183	4/179
Papilloma, Squamous	0/183	1/179
Squamous Cell Carcinoma	0/183	1/179
Adenoma	0/183	1/179
Adenomatous Polyp	0/183	17/179[c]
Papillary Adenoma	0/183	1/179
Bone		
Osteoma	0/183	5/179[d]
Circulatory System		
Hemangioma	6/191	19/190[c]
Hemangiosarcoma	4/191	10/190

[a] Selection based on frequency of occurrence and those possibly exposure related
[b] Number of mice with tumors/Number examined
[c] Statistically higher than controls at $p \leq 0.01$
[d] Statistically higher than controls at $p \leq 0.05$

In several cases there was a statistically significant increase in tumor incidence in exposed mice. Alveolar/bronchiolar adenoma developed in 4 control and 20 UDMH exposed mice. Three alveolar/bronchiolar carcinomas were noted in the exposed, while none were seen in control mice. Twenty exposed and four control mice had hepatocellular adenomas. Only one mouse, exposed to UDMH, had a hepatocellular carcinoma. Thirty-four mice exposed to UDMH developed nasal tissue tumors. The majority of the nasal tumors arose from the epithelial lining while five were induced in the bony tissue of the nasal cavity. None of these forms of nasal tumors were

found in any control mice used in this study. Fifteen of the angiomatous tumors identified in the mice exposed to UDMH occurred in the liver. The incidence of malignant lymphomas in the exposed mice was greater than the incidence in the controls. The frequency in both groups was high since this tumor type is common in aging mice.

A variety of other tumors were found in various tissues of control and exposed animals. The majority of these tumors occurred with equal low frequency in both groups. There was a high frequency of pituitary adenomas in both groups; however, the incidence was significantly higher in the control animals.

SECTION VI

DISCUSSION AND CONCLUSIONS

DISCUSSION

Rats, mice, hamsters, and dogs exposed intermittently for 6 months to selected vapor concentrations of propellant grade UDMH containing trace amounts of DMNA showed no overt signs of toxicity. During exposure, rodent mortality showed no association with UDMH concentration. No dogs died during the exposure phase of the study. For the most part, natural attrition due to aging and associated diseases produced mortality in rodents following exposure. However, spontaneous and UDMH induced tumorigenicity may have accounted for some of the deaths. Body weights of all groups of exposed rats and hamsters were less than their controls during exposure reflecting some stress of UDMH inhalation. The effect, however, was not dose-related. Growth of dogs and mice was unaffected.

Significant non-oncogenic effects in dogs were slight to moderate transitory hepatotoxicity in groups exposed to the 5 ppm concentration. SGPT levels were significantly elevated by the fourth week of exposure and stabilized at levels 3-4 times those of the control dogs throughout the remaining 22 weeks of exposure. BSP retention values were significantly higher than controls at exposure conclusion, but were normal 11 weeks postexposure. SGPT values showed 50% reduction by 2 weeks postexposure and complete recovery at 27 weeks. Hematocrit and RBC values taken immediately postexposure on rats and hamsters showed no abnormalities.

Hamsters and dogs showed no micropathologic effect of UDMH inhalation. Significant changes were seen in various organs of mice and rats. Overall tumor incidence was greater in all exposed rats and mice than in their controls. Malignant tumors of the liver are

of particular importance. In mice exposed to 5 ppm, 16 hemangiosarcomas were found in the livers along with 8 Kupffer cell sarcomas. These findings were statistically significant in all cases. Rats in the high dose group also had a higher incident of bronchiolar adenomas. Pituitary chromophobe adenomas and pancreatic islet cell adenomas were more frequent in rats exposed to the two higher UDMH concentrations than in controls. The various non-neoplastic lung lesions in the 0.5 ppm and 5 ppm exposed mice groups were, for the most part, manifestations of aging. However, these findings suggest that UDMH may have accelerated the aging process. In the case of the rats, the frequency of hyperplastic lesions of the alveolar epithelium in all groups of exposed rats is conspicuous. These lesions are regarded by our pathologists as preneoplastic lesions that may subsequently have developed into bronchiolar adenomas in the high dose group. Cysts occurring in the reproductive organs of old female mice are very common, but the higher incidence of uterine cysts over controls in all groups of UDMH exposed mice may indicate a relationship to exposure.

The data collected from short term exposure of dogs to purified UDMH illustrated that neither intermittent exposure for 8 1/2 weeks nor continuous exposure for 13 days had any hepatotoxic effect in dogs. Moreover, the results of this entire study show that DMNA was the active agent which produced increased SGPT levels in both short and long term exposures and, presumably, BSP changes in the longer term exposures.

Mice exposed intermittently for 12 months to 5 ppm purified UDMH showed no outward manifestation of toxicity. Analytical UDMH concentration control was good and the entire one-year exposure was conducted with no measurable DMNA (less than 35 parts per trillion) in the exposure atmosphere. Mortality was slightly greater in exposed mice than in the control group during the exposure phase of the study, but lacked statistical significance. A reversal of this trend was seen during the postexposure phase, with mortality in both groups equivalent at the end of the study. The stress of 12 months of UDMH inhalation was revealed by lower weight gain in the exposed mice compared with control weights.

There were four primary sites of tumor formation noted in the mice exposed to 5 ppm purified UDMH: lung, liver, nasal cavity, and circulatory system. The majority of the lung and liver tumors were benign adenomas. Malignant tumors in these two organs of purified UDMH exposed mice were not statistically different from control values.

The irritative nature of purified UDMH was evident from the high incidence of inflammatory lesions noted in the nasal mucosa of exposed mice. UDMH is water soluble and was probably absorbed strongly in the nasal portion of the respiratory tract during periods of exposure. Approximately 50% of the mice with suppurative nasal inflammation also had some type of nasal tumor. The majority of these tumors were benign epithelial types, while five nasal bone tumors were noted in the purified UDMH exposed mice. Both of these tumor types are rare in aged mice.

Purified UDMH produced a number of pathologic changes in the circulatory system, both neoplastic and non-neoplastic. The total number of angiomatous tumors was approximately three times greater in the exposed mice compared to the control group. The majority of the angiomatous tumors were found in the liver. Additionally, abnormal dilatation of blood vessels occurred with increased frequency in exposed mice. These lesions were also noted more frequently in the liver of exposed mice.

While some pulmonary carcinomas were seen in mice exposed in the earlier UDMH inhalation study, there was no statistically significant difference noted between the incidences of pulmonary adenomas of control and exposed groups. The longer (one-year) exposure of mice to purified UDMH produced a significant increase in lung adenomas and again showed a few pulmonary carcinomas. Six-months exposure to UDMH failed to produce a statistically significant increase in hepatic lesions over controls. The longer exposure to UDMH (12 versus 6 months) caused significant increases in liver adenomas and hemangiomas. In all, there were 15 hemangiomatous tumors, both benign and malignant, seen in the liver of mice exposed to purified UDMH compared to none in their sham treated controls. Hemangiomatous tumors occurring in other organ systems were comparable in both groups of mice. Malignant lymphoma incidence was increased in the UDMH exposed mice as had been observed in the earlier study. However, the induction of tumors that have high natural occurrences in experimental animals, such as C57BL/6 mice, is less relevant to tumor risk than the induction of tumors that are normally rare in experimental animals.

The current threshold limit value for UDMH is 0.5 ppm (1.0 mg/m^3) (ACGIH, 1981). It has remained unchanged since it was adopted by the ACGIH in 1960. The basis for this TLV was not documented until two years later (1962). Primarily, the work of Jacobson et al. (1955), Hodge (1959), Rinehart (1960), and Shook and Cowart (1957) was used in establishing the TLV for UDMH. The A2 notation (Industrial Substances Suspect of Carcinogenic Potential for Man) appears for the first time in the 1976 TLV's, ACGIH (1976). The NIOSH recommended standard is also 0.5 ppm (1978).

The ACGIH A2 classification requires the demonstration of carcinogenesis in one or more animal species by appropriate methods. The work of Toth (1973) appears to be the basis for the assignment of the A2 notation to UDMH. The TLV Committee provides guidelines for classification of experimental animal carcinogens. There are three classes: high, intermediate, and low potency. In all cases a significant excess of cancers above that occurring in negative controls is required. To qualify as a carcinogen of intermediate potency, a substance should elicit cancer in two animal species at doses intermediate between those described for high and low potency by two routes of administration. The intermediate concentration by inhalation is between 1 mg/m^3 to greater than 10 mg/m^3 and by oral administration is 1 mg/kg/day to 50 mg/kg/day. The results of our inhalation studies show that significant incidences of cancer were produced in two animal species that inhaled a concentration of 5 ppm (12 mg/m^3) UDMH. Toth's oral dosing of mice at 0.01% UDMH in drinking water translates to 28 mg/kg/day. Although qualifying results from oral dosing to another species was not found in the literature, we believe that interpretation of our results together with Toth's would rank UDMH as a substance of intermediate carcinogenic potency in experimental animals.

Insight of the toxicity of DMNA was gained from the result of our studies. Moderate, but transient, hepatotoxicity was demonstrated in dogs exposed for 6-months to 5 ppm UDMH and approximately 6 ppb DMNA. Elevated SGPT and BSP measurements were indications of the effect. The results from a follow-up study with dogs exposed to UDMH with and without DMNA confirmed that DMNA alone was responsible for the hepatotoxic effects in dogs. However, exposure of dogs for 6-months to 0.5 ppm UDMH and approximately 0.6 ppb DMNA caused no alteration in SGPT and BSP values. Thus, an effect and no-effect level has been established for dogs in an organ that is known to be a target for DMNA injury.

Further knowledge was obtained from the results of the investigation of the peroral toxicity of DMNA in CF-1 male mice. Doses of DMNA essentially equivalent to and twice the concentration (5 and 10 µg/kg) of DMNA in the highest concentration (5.0 ppm) of UDMH used in the chronic study failed to produce any significant pathologic indications of hepatotoxicity in mice during and following repeated doses each working day in a 60-day period. A DMNA dose of 50 µg/kg (50 ppb inhalation equivalent) did cause some fatty change and hepatocellular alteration during a 28-day dosing schedule. The degree of damage at 1 mg/kg (1000 ppb equivalent) was judged to be near the point of irreversibility. With due respect to the limited time and dose schedule used in these ancillary dog and mouse experiments, non-tumor effect and no-effect dose levels of DMNA were achieved.

The results of the 6-month exposure of multiple species to unpurified UDMH together with information from our additional studies suggest that 6 ppb is a minimum effect level for DMNA toxicity in animals by the inhalation route. A concentration between 0.6 and 6 ppb may be an absolute no-effect level, but definitive experiments at very low concentrations have never been conducted by other investigators by any route of administration.

The problem of DMNA contamination in the production of UDMH is an important factor in the consideration of the toxicity and oncogenicity of UDMH. If supplies for industrial and military use contain no more than 0.12% DMNA, then the results of our studies can be used in evaluating safe exposure levels for humans involved in the production and use of UDMH. Under these conditions we believe that the current TLV of 0.5 ppm UDMH is a safe level for man. This includes non tumor effects and consideration of cancer risk. Manufacturing and use of UDMH with DMNA content in excess of 0.12% should be scrutinized carefully with regard to the potential oncogenic capability of DMNA even though tumor production in humans has never been documented.

The results of our studies have filled the void in the experimental determination of definitive UDMH oncogenic and nononcogenic inhalation dose response studies. Significant knowledge of the toxicity of DMNA was also gained as a result of this investigation.

CONCLUSIONS

The results of the 6-month study provided evidence that propellant grade UDMH was tumorigenic in rats and mice mainly at the highest concentration level tested. Tumors were not produced in hamsters and dogs from UDMH inhalation. The liver was the main target of UDMH induced pre-neoplastic and neoplastic changes in rats and mice with important lesions occurring in the lungs of rats as well. An indication of hepatotoxicity in dogs was demonstrated by transitory elevations of serum enzyme levels and liver function values in animals exposed to 5 ppm UDMH.

This study has shown that the tumor incidence observed in mice exposed to propellant grade UDMH for a shorter (6-month) period of time was definitely a direct response to the UDMH and not the result of contamination by DMNA. The effect of increasing the exposure phase of the study from 6 to 12 months duration resulted in a significantly increased oncogenic response to the purified UDMH. This was particularly true in the induction of lung adenomas which were

not significantly elevated above controls after the 6-month exposure and of nasal tumors and liver adenomas which were not seen at all previously.

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